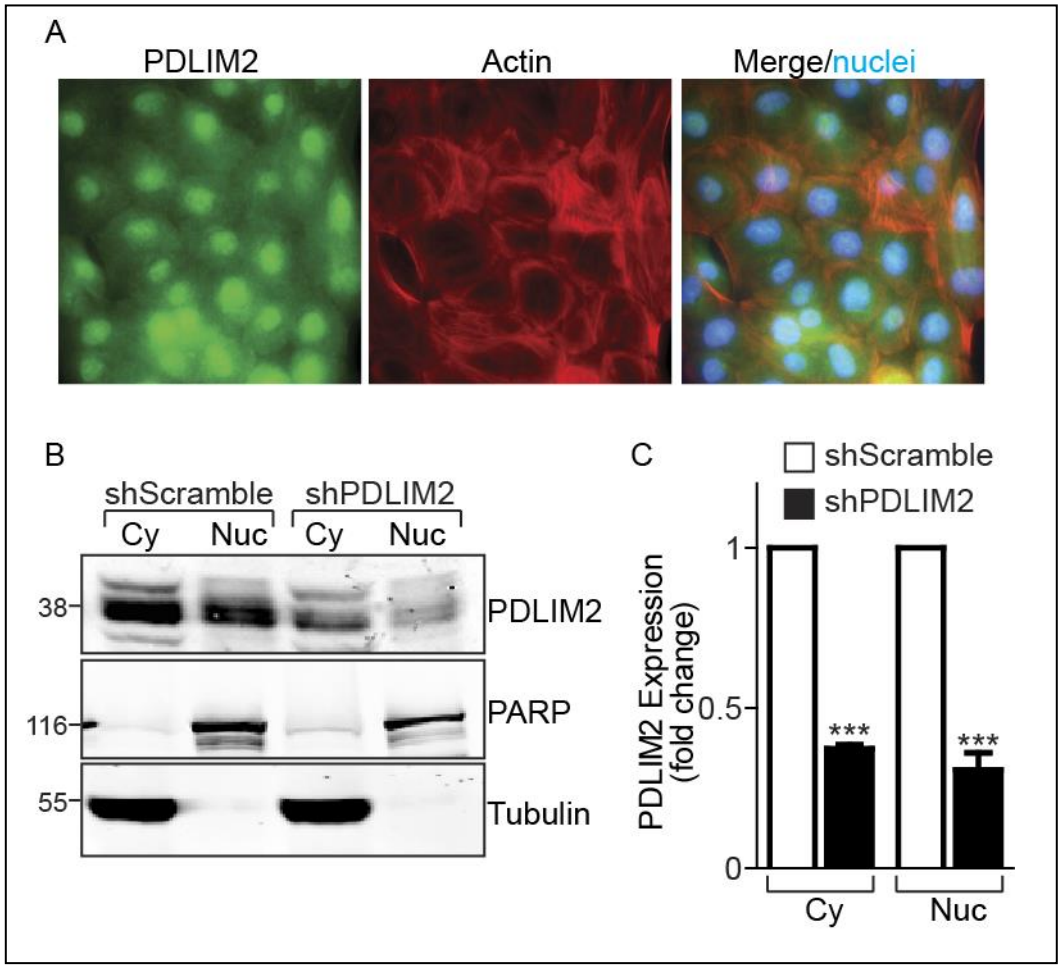
SUPPLEMENTAL DATA

Supplemental Figure S1

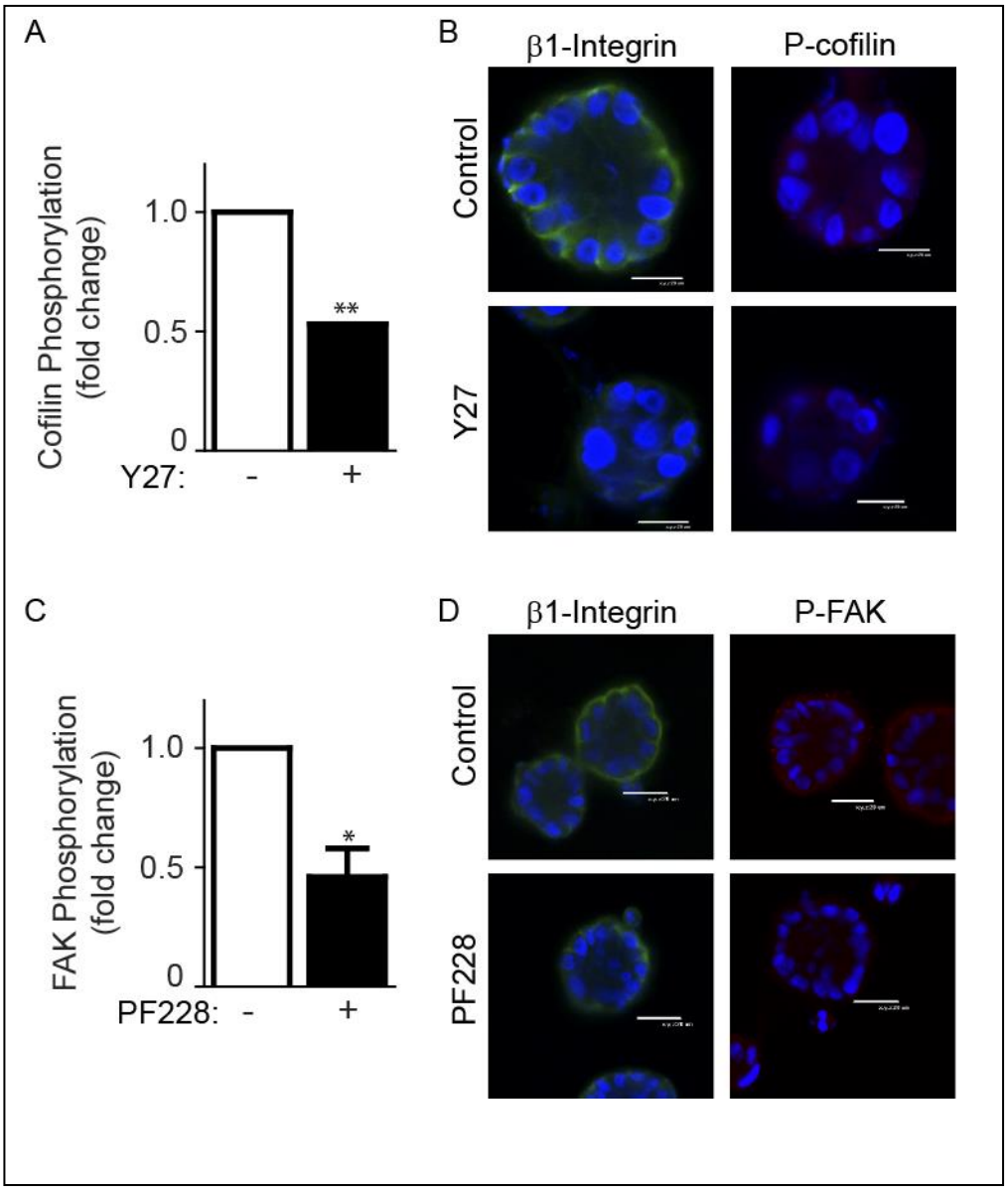
Suppression of PDLIM2 expression is evident in the nucleus and cytoplasm of MCF10A cells stably expressing shRNA targeted to PDLIM2.



(A) Parental MCF10A cells were grown to confluency, fixed and assessed for PDLIM2 expression (green) by immunofluorescence as described in supplemental methods. The actin cytoskeleton was stained with TRITC-phalloidin (red), nuclei were detected using Hoechst dye (blue). **(B)** shScramble and shPDLIM2-MCF10A cells were cultured overnight in complete medium. Cytoplasmic and nuclear fractions were prepared from cells as described in supplemental methods. **(C)** Densitometric quantification of PDLIM2 expression from three separate experiments are shown with data presented as ±SEM, n=3, ***P<0.0005.

Supplemental Figure S2

Inhibition of the FAK/Rho Kinase pathway suppresses signalling downstream of β1 integrin in control (shScramble) MCF10A cells.



(A) Graph showing fold change of cofilin phosphorylation in shScramble MCF10A cells, following ROCK inhibition (Y27; 10μM, 24hr), quantified by densitometry. The data are presented as mean ± SEM from 3 separate experiments. **(B)** ShScramble MCF10A cells were cultured in a 3D Matrigel assay for 12 days in the presence or absence of ROCK inhibitor (Y27; 10μM) or DMSO vehicle (control). Cell structures were fixed and processed for confocal microscopy analysis for β1-integrin (green) and P-cofilin (red) expression.

Representative confocal midplane sections are shown, nuclei are blue. **(C)** Graph showing fold-change of FAK phosphorylation in shScramble MCF10A cells, following FAK inhibition (PF228; 50nM, 24hr), quantified by densitometry. The data are presented as mean ± SEM from 3 separate experiments. **(D)** ShScramble MCF10A cells were cultured in a 3D Matrigel assay for 12 days in the presence or absence of FAK inhibitor (PF228; 50nM) or DMSO vehicle (control). Cell structures were fixed and processed for confocal microscopy analysis for β1-integrin (green) and P-FAK (red) expression. Representative confocal midplane sections are shown, nuclei are blue. Data are representative of 3 separate experiments with similar results.

Supplemental Methods;

Cytoplasmic and Nuclear Fractionations

Cellular fractionations were performed as described by Lau et al, with amendments (Lau et al., 2007). Briefly, cells were lysed with cytoplasmic lysis buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10% glycerol, 1 mM DTT, 0.1% Triton X-100 and protease inhibitors (as described in methods) and incubated on ice for 7 min. Lysates were centrifuged at 4000 rpm at 4°C for 5 min, the supernatant was collected (cytoplasmic fraction) and the nuclear pellet was resuspended in nuclear lysis buffer (0.2 mM EGTA (pH 8), 3 mM EDTA (pH 8), 1 mM DTT) and vortexed overnight at 4°C. Samples were centrifuged at 4000 rpm for 5 min at 4°C and supernatant collected (Nuclear fraction). Protein concentration was determined from cytoplasmic fractions, extrapolated to nuclear fractions, and 100µg of protein from each fraction was resolved on a 10% acrylamide gel. PARP antibody was from Cell Signalling technology, (Beverly, MA).

Immunofluorescence staining of 2D cell cultures

Cells were fixed and stained using PDLIM2 antibody and TRITC-phalloidin as described previously (Bowe et al., 2014).

BOWE, R. A., COX, O. T., AYLLON, V., TRESSE, E., HEALY, N. C., EDMUNDS, S. J., HUIGSLOOT, M. & O'CONNOR, R. 2014. PDLIM2 regulates transcription factor activity in epithelial-to-mesenchymal transition via the COP9 signalosome. *Mol Biol Cell,* 25**,** 184-95.

LAU, L., HANSFORD, L. M., CHENG, L. S., HANG, M., BARUCHEL, S., KAPLAN, D. R. & IRWIN, M. S. 2007. Cyclooxygenase inhibitors modulate the p53/HDM2 pathway and enhance chemotherapy-induced apoptosis in neuroblastoma. *Oncogene,* 26**,** 1920-31.